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Hepatitis C Virus Infection and Genotypes in Southern Israel and the Gaza Strip

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The Gaza Strip borders the southern part of Israel and Egypt. There is a remarkable difference in the prevalence of antibodies to hepatitis C virus (HCV) between Israel (0.5%) and Egypt (10%). A few thousand inhabitants cross the borders daily from the Gaza Strip to both countries. The objectives of this study were to investigate the prevalence of HCV infection in the Gaza Strip, an area that was not studied before, and to study HCV transmission in the Gaza Strip by characterizing the genotypes of HCV in Southern Israel and the Gaza Strip and comparing them with those found in Egypt. HCV prevalence in the Gaza Strip was found to be 2.2%, relatively higher than in Israel but lower than in Egypt. The most common genotypes found were type 1b in Southern Israel and type 4 in the Gaza Strip, corresponding to the most prevalent genotype in Egypt. Similarity between type 4 isolates from the Gaza Strip and Egypt was illustrated further by sequence analysis of the HCV 5′ noncoding region (NCR). ***J. Med. Virol. 56:230–233, 1998.***

KEY WORDS: HCV; genotype; prevalence; PCR

INTRODUCTION

Worldwide, hepatitis C virus (HCV) is a major etiologic agent of chronic hepatitis that may lead to the development of liver cirrhosis and hepatocellular carcinoma. Parenteral transmission, in recipients of blood and blood products, intravenous drug abusers, and dialysis and renal transplantation patients, accounts for 60–70% of HCV infections [Alter, 1995]. A small percentage of cases is due to intrafamilial and mother-to-infant transmission. However, in 25% of the cases the origins of HCV infections are unknown [Alter et al., 1992; Alter, 1993]. Hence studies of the infection routes are needed in order to implement effective control measures against the spread of HCV.

Based on nucleic acid sequence analysis, six major genotypes and more closely related subtypes have been identified [Simmonds et al., 1994]. Genotypes 1, 2, and 3 are widely distributed throughout Western countries and Asia. Types 5 and 6 are largely confined to South Africa and Southeast Asia, respectively, whereas type 4 is found predominantly in the Middle East and central Africa [Bukh et al., 1993; Hibbs et al., 1993; McOmish et al., 1994]. The reason for the difference in the geographical distribution of HCV types is not known. Certain risk factors, or difference in the mode of transmission, may be involved in determining patterns of genotype predominance. The different genotypes can be used to study transmission routes in areas with distinct genotype pattern.

This is a report of the status of HCV infection in the southern part of Israel and the neighboring area of the Gaza Strip, areas that have not been investigated previously. The Gaza Strip is located between the south of Israel and Egypt, and a few thousand inhabitants cross the border daily from the Gaza Strip to both countries. This area is a crossroads between two regions with different HCV prevalence rates and different genotypes. While in Israel HCV prevalence is low (0.5%), in Egypt the infection occurs at high incidence (10%). Studies have shown that the major genotype in Egypt is type 4 [Saeed et al., 1991; Simmonds et al., 1994]. The aim of this study was to determine the prevalence of HCV antibodies and genotypes in the Gaza Strip and southern Israel.

MATERIALS AND METHODS

Study Population in the Gaza Strip

In the Gaza Strip the study population consisted of two groups: blood donors and patients attending the Khan-Yunis hospital.

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Blood donors. The total number of blood donations in the Gaza Strip on average in 1992 was 11,000 per year (Z. el Astal, personal communication). About 99% of blood donors are males. Three blood banks serve the entire area. Two of these blood banks are associated with the two general hospitals in Gaza and in Khan-Yunis. The cohort described here included sera samples collected from 1,509 consecutive blood donors from the Khan-Yunis hospital blood bank, which were collected during a period of 6 months.

Patients. Sera samples from 124 patients attending the various outpatient clinics of the Khan-Yunis hospital were collected at random during a 3-month period. The available clinical data for these patients were very limited. In addition, four serum samples were collected from hemodialysis patients.

Study Population in Southern Israel

Sera from 74 consecutive anti-HCV–positive patients attending the Liver Clinic, Soroka Medical Center, Beer-Sheva, were collected. Detailed clinical data were available from their medical records.

Anti-HCV Screening

In the Gaza Strip, anti-HCV was determined on freshly collected serum samples using INNO-LIA HCV Ab. second and third generation (Innogenetics, Antwerp, Belgium). In Israel, anti-HCV was determined using an IMX apparatus according to the manufacturer's instructions (Abbott Diagnostics Laboratories, North Chicago, IL). HCV seropositive sera were kept in −70°C until studied further.

RT-PCR for HCV Genome

Total RNA was extracted from 200 μl of sera using the QIAamp blood kit (QIAGEN GmbH, Germany) and eluted from the column with 50 μl of water containing 40 U of RNase Blocker I (Promega, Madison, WI). Reverse transcription (RT) of the 5′ noncoding region (NCR) of HCV RNA was performed with HCV antisense oligonucleotide (position 604–586) and 13 μl of RNA. The cDNA was amplified with Taq enzyme by the polymerase chain reaction (PCR) using primers from positions 27–48 (5′ primer) and 348–329 (3′ primer). Confirmation of the PCR product was carried out by Southern blot hybridization with a fourth primer (position 306–287).

In HCV RNA-positive samples, HCV genotypes were determined by three different methods. (1) In restriction fragment length polymorphism (RFLP), positive samples in the first round of PCR were further amplified by nested PCR (using primers from position 51–72 at the 5′ and position 306–287 at the 3' end) and then subjected to RFLP as described by Davidson et al. [1995] using restriction enzyme *Hae*III + *Rsa*I, *Bst*NI + *Hin*fI for genotype analysis and *Bst*UI + *Scr*fI for subtyping of genotype 1 and 2. (2) In sequence analysis, the total PCR product was purified by Wizard PCR prep purification system (Promega) according to the manufacturer's recommendation. Sequencing was done by the Taq dideoxy terminator cycle sequencing method on a 373A DNA sequencer of Applied Biosystems. The 5′ primer at position 27 served as sequencing primer. In 10 cases the amplified sequence of the 5′ NCR was subcloned into the TA vector (InVitrogen, Leek, Netherlands) and sequenced with 3'

TABLE I. Prevalence of Anti-HCV Antibodies and HCV RNA in Khan Yunis[a]

	Blood donors	Renal failure	Ambulatory patients
Total number tested	1,509	4	124
HCV seropositives (% of total)	34 (2.2)	4	11 (8.8)
HCV RNA-positive (% of seropositives)	24 (71)	4	7

[a]The groups evaluated for the presence of anti-HCV antibodies and HCV RNA were blood donors, ambulatory patients attending the hospital for different reasons, and four renal failure patients.

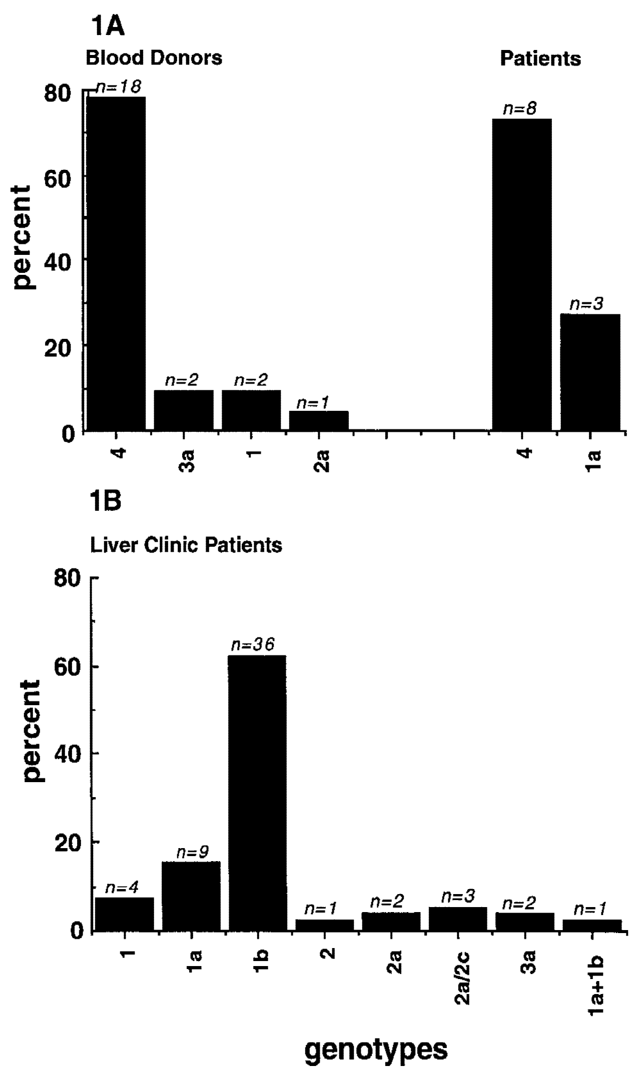

Fig. 1. Distribution of HCV genotypes among blood donors and patients in the Gaza Strip (**A**) and among patients of the Liver Clinic, Soroka Medical Center (**B**).

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                10         20         30         40         50         60
                 |          |          |          |          |          |
EG-1    gttagtatga gtgttgtaca gcctccagga cccccccctcc cgggagagcc atagtggtct
G-8            --- ---------- ---------- ---------- ---------- ----------
G-93    ---------- ---------- ---------- ---------- ---------- ----------
G-220   ---------- ---------- ---------- ---------- ---------- ----------
G-191   ---------- ----c--g-- ---------- ---------- ---------- ----------
G-12    ---------- ----c--g-- ---------- ---------- ---------- ----------
G-14    ---------- ----c--g-- ---------g ---------- ---------- ----------
G-19    ---------- -------g-- ---------- ---------- ---------- ----------

                70         80         90        100        110        120
                 |          |          |          |          |          |
EG-1    gcggaaccgg tgagtacacc ggaatcgccg ggatgaccgg gtcctttctt ggattaaccc
G-8     ---------- -----g---- ---------- ---------- ---------- ----c-----
G-93    ---------- ---------- ---------- ---------- ---------- ----t-----
G-220   ---------- ---------- ---------- ---------- ---------- ----------
G-191   ---------- ---------- ---------a ---------- ---------- ----n-----
G-12    ---------- ---------- ---------- ---------- ---------- ----t-----
G-14    ---------- ---------- ---------- ---------- ---------- ----t-----
G-19    ---------- ---------- ---------- ---------- ---------- ---c------

               130        140        150        160        170        180
                 |          |          |          |          |          |
EG-1    cgctcaatgc ccggaaattt gggcgtgccc ccgcgagacg gctagccgag gagttttggg
G-8     ---------- ---------- ---------- ---------t ---------- t---g-----
G-93    --g------- ---------- ---------- ---------- ---------a ---------t
G-220   ---------t ---------- ---------- ---------- ---------- ----------
G-191   ---------- -t---g---- ---------- ----a---t- ---------- ----------
G-12    ---------- ---------- ---------- ----a----- ------t--- ----------
G-14    ---------- ---------- ---------- ----a----- ---------- ----------
G-19    ---------- ---------- ---------- ----a----- ---------- ----------
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Fig. 2. Layout of HCV 5′ NCR genome, isolated from seven sera with genotype 4, Gaza Strip (G), aligned with a representative of published sequences of genotype 4 isolated in Egypt (EG) [Simmonds et al., 1994].

and 5′ primers, T7 and SP6, using a sequenase kit (United States Biochemical, Cleveland, OH). (3) In Inno-Lipa, part of the samples were amplified by PCR and subjected to genotyping using the Inno-Lipa kit (Innogenetics) according to the manufacturer's instructions.

Biochemistry

Twenty-three sera of anti-HCV–positive blood donors in Khan-Yunis were tested for aspartate aminotransferase (AST) using the standard method described by Bauer [Bauer et al., 1974]. Normal values for adults are 8–38 U/L.

Statistics

The results are expressed as mean ± SD. Comparison between means was carried out using the Student's *t*-test for unpaired data.

RESULTS

HCV Infection in the Gaza Strip

Of the 1,509 blood donors, 34 (2.2 %) were found positive for anti-HCV (Table I). HCV-RNA was detected in 24 (71%) of the seropositives. Of the 124 nonhospitalized patients, 11 (9%) had anti-HCV and HCV RNA was detected in seven (64%) of the seropositives. All of the four hemodialysis patients were anti-HCV– and HCV RNA-positive.

The mean age of anti-HCV–positive blood donors was significantly higher than that of anti-HCV–negative blood donors (38 ± 15 vs. 30 ± 9 years; $P < 0.05$). Twenty-eight anti-HCV–positive blood donors could be traced back and questioned, and only two had a past history of blood transfusion. Twenty-three sera samples from anti-HCV–positive blood donors were available for aspartate aminotransferase determination. The mean AST level in HCV RNA-negative subjects (n = 7) was 18 ± 10 IU/L, (range 18–38). The mean AST level in HCV RNA-positive samples (n = 16) was 33 IU/L, (range 17–63), significantly higher ($P <$ 0.01) than the value observed in the RNA-negative group.

HCV Infection in Southern Israel

Out of the 74 anti-HCV–positive sera, 60 (81%) had HCV RNA. In the HCV RNA-positive group, the clinical manifestations were 58% chronic hepatitis, 28% cirrhosis, 6% hepatocellular carcinoma (HCC), and 8% normal liver enzymes. In the HCV RNA-negative group, six (43%) had normal liver enzymes, one had chronic hepatitis, three had cirrhosis, two had HCC, and two had autoimmune hepatitis.

HCV Genotypes in the Gaza Strip and in Southern Israel

In the Gaza Strip 23 samples were available for genotype analysis. Genotype 4 was detected in 18 donors (78%). Two donors were genotype 3a, two were genotype 1, and one had genotype 2a (Fig. 1A). The persons with genotypes 1a and 3a reported to have spent long years out of the Gaza Strip area: in Jordan, Libya, Saudi Arabia, and Israel.

Of the 7 nonhospitalized patients, six had genotype 4 and one had genotype 1a. Of the four hemodialysis patients, two had genotype 4 and two genotype 1a.

In Southern Israel, the main genotype found was type 1b (62%). The rest of the genotypes were 1a (15%), 1 (7%), 2, 2a and 2a/2c (10%), and 3a (3.5%) (Fig. 1B). Genotype 4 was not detected in any of the Israeli samples.

In order to determine the genetic distance to Egyptian genotype 4 isolates [Simmonds et al., 1994], the sequences obtained from 5′ NCR of seven randomly selected genotype 4 isolates from the Gaza Strip were aligned. No major differences were detected (Fig. 2).

DISCUSSION

This is the first report concerning the prevalence of antibodies to HCV in blood donor population and HCV genotypes in the Gaza Strip and southern Israel. The prevalence of anti-HCV among blood donors in the Gaza Strip was 2.2% (Table I), five times higher than in Israel but four times lower than in Egypt. The most prevalent genotype of HCV in the Gaza Strip among blood donors and nonhospitalized patients was type 4, resembling the most abundant type in Egypt [Simmonds et al., 1994]. However, in southern Israel 60% of the patients attending the Liver Clinic had genotype 1b, while genotype 4 was not found among them. The high similarity between the Gaza Strip and Egypt isolates was further demonstrated when the 5′ NCR sequences of genotype 4 viral DNAs isolated from the Gaza Strip were compared with type 4 isolates from Egypt. No significant sequence differences were detected (Fig. 2). The other genotypes detected in the Gaza Strip were types 1, 2a, and 3a in the blood donor population and types 3a and 1a in patients. Similar genotypes were found in Israel. In the blood donor group, the history of 28 HCV-positive persons could be ascertained. Of these, only two had reported to have been exposed to possible source of HCV infection, e.g., blood transfusions, while for the remaining 26, no obvious possible source of infection could be found. This leaves the possibility of infections through other risk factors, e.g., intrafamilial exposure and contaminated syringes.

HCV genotype analysis within a defined population provides a useful technique to compare infection and transmission in different geographical regions and risk groups. The movement of population into the Gaza Strip is well documented. The region was inhabited by the Palestinian people in 1948 and was, until 1967, under Egyptian administration. Between 1967 and 1993, it was under Israeli administration. The area is situated on the border between Israel and Egypt. In Egypt, the rate of HCV seropositives among the unpaid blood donor population is 10% [Saeed et al., 1991] and the most common genotype of HCV is type 4 [Hibbs et al., 1993; Simmonds et al., 1994], while in Israel the prevalence of anti-HCV in blood donors is only 0.5% and the genotype pattern consists of the types 1 to 3, with the most prevalent (more than 70%) being type 1b (Fig 1 and the preliminary data of Bogomolski-Yahalom et al. [1997]).

Our results hint at the period of HCV transmission in the studied population (between 1948 to 1967) and might give an indication that at that time, genotype 4 was already most prevalent in Egypt. Furthermore, the age difference between anti-HCV–positive and –negative blood donors raises the question whether certain past health management practices, e.g., vaccinations, may have influenced HCV infection rate and transmission. Further epidemiological/serological studies of different age groups in the Gaza Strip are needed to clarify these issues.

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